The role of two eulophid parasitoids in populations of the leafminer, *Phyllonorycter mespilella* (Lepidoptera:Gracillariidae) in British Columbia

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ABSTRACT

In 1991, orchards in the Naramata region of the Okanagan valley in British Columbia had significantly larger populations of the leafminer, *Phyllonorycter mespilella*, than those of the Osoyoos/Oliver region, 50 km south, where parasitism had been shown to keep the miner below treatment levels. We questioned if different roles of the parasitoid species caused the discrepancy. Leaves were collected and leafminers and parasitoids assessed from overwintering populations and also weekly from May through October (1992) in apple orchards representative of the areas. *Pnigalio flavipes* and *Sympiesis marylandensis* were the major parasitoid species overwintering in 52.3 and 46.7% respectively of the *P. mespilella* mines. The percentages of the two species did not differ significantly between the orchards screened in both areas and did not account for the differences in the numbers of overwintering or summer generation mines. *P. flavipes* was the dominant parasitoid species in both regions through the three summer generations. *S. marylandensis* was only found at low levels in three of the eight orchards until the second and third generations. Parasitoid-induced-mortality in 1992 did not have a consistent significant impact on intraseasonal leafminer increase. Five of the orchards studied had leafminer populations above treatment thresholds.

Key words: Treefruit, biological control, Hymenoptera, leafminer

INTRODUCTION

Phyllonorycter mespilella (=elmaella) (Hubner) (Lepidopera: Gracillariidae), is a tentiform leafminer that feeds within the leaves of several economically important fruit trees in the Pacific Northwest (Hoyt 1983). Four parasitoid species were associated with this leafminer when it was first established in the orchards of the Okanagan and Similkameen valleys of British Columbia in 1988 (Cossentine and Jensen 1992). *Pnigalio flavipes* (Ashmead) (Hymenoptera: Eulophidae) was identified as the major parasitoid of the leafminer in British Columbian and Washington State orchards (Barrett 1988, Cossentine and Jensen 1992). Parasitism by this species could reduce the host's intraseasonal population increase and keep its density below treatment levels (Barrett and Brunner 1990). The value of the other three leafminer parasitoids, species of *Sympiesis, Eulophus* and *Cirrospilus* (Hymenoptera: Eulophidae), in control of *P. mespilella* in the interior of British Columbia have not been studied.

In the 1991 apple growing season, orchards in the Naramata area of the Okanagan valley had very high populations of the leafminer. This was in contrast to the well controlled populations of *P. mespilella* in the Osoyoos and Oliver orchards, about 50 km further south where *P. flavipes* was first established, and its effective role in regulating the leafminer populations during the summer became evident (Cossentine and Jensen 1992). In the 1992 growing season we sought to determine if the parasitoids and their roles differed between the two areas, and if these differences could explain the variation in the leafminer populations.

MATERIALS AND METHODS

In 1992 we selected five orchards in the Naramata area that had high populations of *P. mespilella* in 1991 and three in the Oliver/Osoyoos area which had low populations that had been well controlled by parasitism in the past. More orchards and leaves were screened in Naramata, because judging from high leafminer counts in previous years, we expected fewer parasitoids would be found.

We collected leaves in January 1992 (Naramata 200, Oliver/Osoyoos 100) from the orchard floor at each site before winter diapause of the leafminer or parasitoid pupae was broken. Leaves from each orchard were placed in a ventilated 2-l plastic container and held at room temperature (20-22°C). The containers were checked daily for parasitoid emergence and the adult parasitoids were frozen until identified and sexed. Representative specimens were identified by systematists at the Centre for Land and Biological Resources Research, Ottawa. Comparisons of emerging parasitoids were based on the assumption that each species would have an equal chance of successfully developing under the described conditions. The leaves from three orchards in Naramata were so severely torn that mines per leaf could not be assessed. An estimated appropriate mass of torn leaves was therefore collected from each of these orchards so that emerging parasitoid adults could be collected and identified.

One hundred leaves were collected randomly from the canopy of each orchard weekly from May 7 until October 5, and on October 24. The infestation level was determined by counting the mines per leaf and assessing the mine contents of these leaves. This count included the numbers of living and dead sap-feeders (instars 1-3) and tissue-feeders (instars 4-5), pupae, emerged pupae, parasitoid eggs, larvae and pupae, mines with a round parasitoid exit hole, as well as empty mines. Leaves containing parasitoids in any stage of development were placed in ventilated 2-l plastic containers and the emerging parasitoid adults were aspirated, frozen and later identified and sexed.

Pnigalio and *Sympiesis spp.* cause leafminer mortality both as larvae which feed externally on host larvae as well as adults 'host-feeding' on leafminer larvae and occasionally on pupae (Barrett 1988, Van Driesche and Taub 1983). In the first generation total parasitoid-induced-mortality (PIM) was assessed as the number of mines containing dead leafminers and/or parasitoids divided by the total number of mines minus the number of empty mines. The fate of the *P. mespilella* from empty mines could not be determined. In the second and third generations the PIM and total number of mines per leaf were assessed as above; however, mines with emerged pupae, empty mines and parasitoid exit holes were removed from the calculations because they may have occurred in the first generation. The number of parasitoids emerging per mine was de-

Table 1

Phyllonorycter mespilella mines per overwintered apple leaf and for each summer generation, assessed on 100 leaves at peak sap- and tissue-feeding stages.

	Mines per apple leaf						
	Overwintering	First Generation		Second Generation		Third Generation	
	(n)	sap	tissue	sap	tissue	sap	tissue
Naramata Orchards							
1.	11.00 (200)	0.07	0.06	1.07	1.84	4.82	7.48
2.	5.57 (200)	0.36	0.03	1.88	3.55	5.32	5.78
3.	[a]	0.03	0.02	0.41	1.79	6.82	7.53
4.	[a]	0.00	0.00	0.08	1.03	2.46	6.54[b]
5.	[a]	0.19	0.15	2.02	4.18	9.33	13.94
Osoyoos/Oliver Orchards							
1.	2.76 (100)	0.10	0.06	2.71	4.25	14.64	15.64
2.	0.44 (100)	0.02	0.02	0.24	0.58	2.90	2.82
3.	1.22 (100)	0.02	0.01	0.75	1.76	7.63	8.10

[a] leaves were torn and mines per leaf could not be determined.

[b] count from previous week, orchard cut down.

Table 2

Pnigalio flavipes (Pf) and *Sympiesis marylandenis* (Sm) parasitoids per *Phyllonorycter mespilella* mine (n=total mines).

	Parasitoids per mine per parasitoid generation														
	Overwintering			First			Second			Third			Fourth		
	Pf	Sm	n	Pf	Sm	n	Pf	Sm	n	Pf	Sm	n	Pf	Sm	n
Naramata Orchards															
1	0.12	0.08	2222	0.19	0.00	57	0.08	0.03	73	b	b	50	0.10	0.00	136
2	0.10	0.09	1114	0.12	0.02	73	0.36	0.10	125	b	b	50	0.05	0.00	215
3	a	a		0.07	0.00	61	0.17	0.00	123	0.06	0.02	354	0.03	0.03	254
4	a	a		0.00	0.00	50	0.07	0.07	101	b	b	50	0.06	0.06	156[c]
5	a	a		0.49	0.00	114	0.07	0.00	109	0.03	0.00	371	b	b	50
Osoyoos/Oliver Orchards															
1	0.11	0.13	276	0.18	0.00	61	0.37	0.07	159	0.14	0.00	442	0.03	0.03	375
2	0.00	0.12	44	0.24	0.03	50	0.46	0.00	89	0.26	0.00	92	0.18	0.00	58
3	0.28	0.05	122	0.17	0.02	53	0.29	0.06	121	0.26	0.00	171	0.10	0.00	219

[a] leaves were torn and mines could not be determined.
[b] no parasitoid adults emerged to identify.
[c] counts from previous week, orchard cut down.

Table 3

Mortality attributed to parasitoids (PIM) per 100 apple leaves at peak tissue-feeding *Phyllonorycter mespilella* stages within each of three summer generations.

	Mortality[a]		
	First Generation	Second Generation	Third Generation
Naramata Orchards			
1.	38.6[b]	87.9	96.8
2.	32.9	72.7	100.0
3.	8.2	47.0	99.2
4.	50.0	34.4	79.5
5.	75.8	86.7	99.5
Osoyoos/Oliver Orchards			
1.	19.7	95.3	87.0
2.	42.0	84.2	94.6
3.	30.2	80.5	99.3

[a] Mortality was considered to include: parasitoid eggs, larvae, pupae, parasitoid exit holes (first generation only) and dead host larvae and pupae divided by total mines (excluding mines containing emply pupae, parasitoid exit holes or empty mines in the second and third generations).

[b] each value in the table represents the assessment per 100 mines.

termined as the total of adult parasitoids divided by the number of mines per leaf assessed as above.

Parasitoid and leafminer counts were not averaged over weeks within generations because each sample could not be considered a replicate of an extended stabilized point in the host-parasitoid life histories (Van Driesche 1983). Rather, the leafminer populations were compared at five individual weeks during maximum sap-feeding and tissue-feeding stages within each generation. PIM was assessed at the peak tissue-feeding stage when conditions were most opportune for parasitism and host-feeding.

P. mespilella mines per leaf, parasitoids per mine and PIM were compared between the two regions using an ANOVA (SAS Institute 1985). Percent parasitism, the sex of each parasitoid, mines per leaf and PIM within the overwintering population were compared between the two regions using a correlation procedure (SAS Institute 1985).

RESULTS AND DISCUSSION

Overwintering population. Leaves collected in January from orchards in the Naramata area contained significantly (p=0.04) higher numbers of mines per leaf than did leaves collected from Oliver and Osoyoos orchards (Table 1). This illustrates the contrasting infestation levels between the two orcharding regions observed in 1991. Emerging total number of overwintering parasitoids per mine did not differ significantly (p>0.05) between the two regions (Table 2).

P. flavipes and *Sympiesis marylandensis* Girault (Hymenoptera: Eulophidae) (identified by J. Huber, Centre for Land and Biological Resources Research, Ottawa) were the parasitoid species most commonly reared from overwintering *P. mespilella* mines. *S. marylandensis* is probably the same species of *Sympiesis* previously recorded as a minor parasitoid of the leafminer in the Okanagan valley (Cossentine and Jensen 1992), although it is not one of the three species of *Sympiesis* previously cited as parasitizing *Phyllonorycter elmaella* in the Fraser valley (Doganlar and Bierne 1980).

P. flavipes represented 52.3% and *S. marylandensis* 46.7% of the overwintering parasitoid populations in the eight orchards (n=358). This was unexpected as *P. flavipes* was the dominant parasitoid species in the summer studies of 1988-1990 (Cossentine and Jensen 1992) and was therefore expected to infest a higher proportion of the overwintering mines. Percentage of the total population varied from 0 to 85.7% and from 14.3 to 100% for *P. flavipes* and *S. marylandensis*, respectively. The percentages of *P. flavipes* and *S. marylandensis* in the overwintering mines did not differ significantly ($p>0.05$) between the two regions. Barrett and Jorgensen (1986) reported that *S. marylandensis* was the dominant parasitoid species of the first and third *P. elmaella* generations in Utah and that *P. flavipes* was dominant in the second and fourth generations. The percentages of male and female adults of each of the two parasitoid species did not differ significantly ($p>0.05$) between the two regions (*Pnigalio* male:78.5%, 61.9%; female: 21.5%, 38.2%; *Sympiesis* male: 78.7%, 90.5%; female: 21.3%, 9.5%, in the Naramata and Osoyoos/Oliver regions, respectively).

Zagrammosoma multilineatum (Ashmead) (Hymenoptera:Eulophidae), a species not previously recorded from *P. mespilella* in British Columbia (Cossentine and Jensen 1992, Doganlar and Bierne 1980) represented 6.06% of the parasitoid population emerging from one Naramata

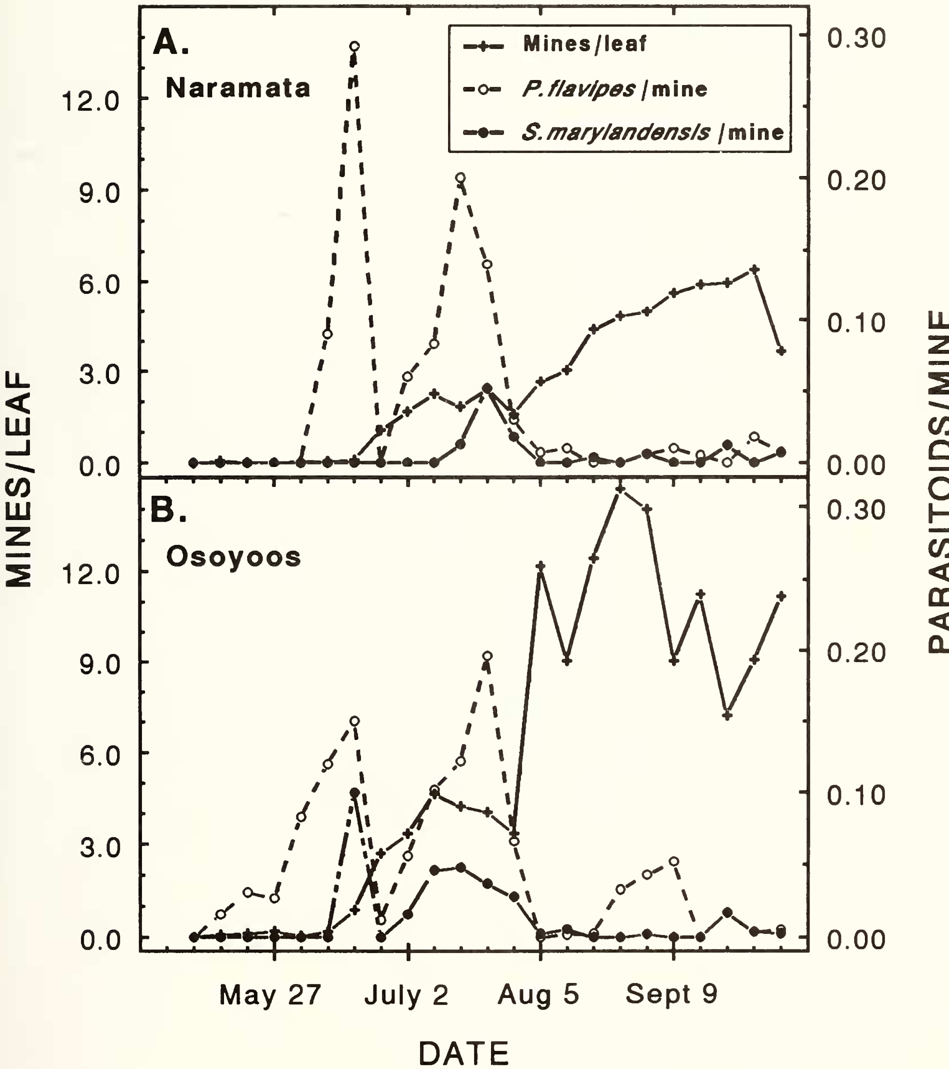

Figure 1. *Phyllonorycter* mines per leaf and parasitism by *Pnigalio* and *Sympiesis* per mine from May to October 1992. A=orchard 1, Naramata; B = orchard 1, Osoyoos/Oliver.

orchard. Over all orchards this represented only 0.76% (n=385) of the overwintering parasitoids.

Summer populations. The number of sapfeeding mines per leaf did not differ significantly (p>0.05) between the two regions in the first generation (Naramata: $\bar{x}$=0.13 mines/leaf, Oliver/Osoyoos: $\bar{x}$=0.05 mines/leaf) (Table 1). We then questioned if the percent parasitism by each species independently, or the sex ratio of *P. flavipes* or *S. marylandensis* within the overwintering population, affected the number of *P. mespilella* mines per leaf or percent of PIM in the first generation. None of these factors in the overwintering population consistently or significantly (p>0.05) influenced mines per leaf or PIM in the first generation when counts were analyzed as separate regions and as a whole (Tables 1 and 3). The number of overwintering mines was not significantly correlated with the number of sap-feeding (r=0.2, p=0.63)) or tissue-feeding (r=0.65, p=0.23) mines in the first generation. This contradicts Barrett and Brunner's (1990) conclusion that the survival of overwintering leafminers, rather than the seasonal levels of parasitism, determine the leafminers' year-to-year population dynamics.

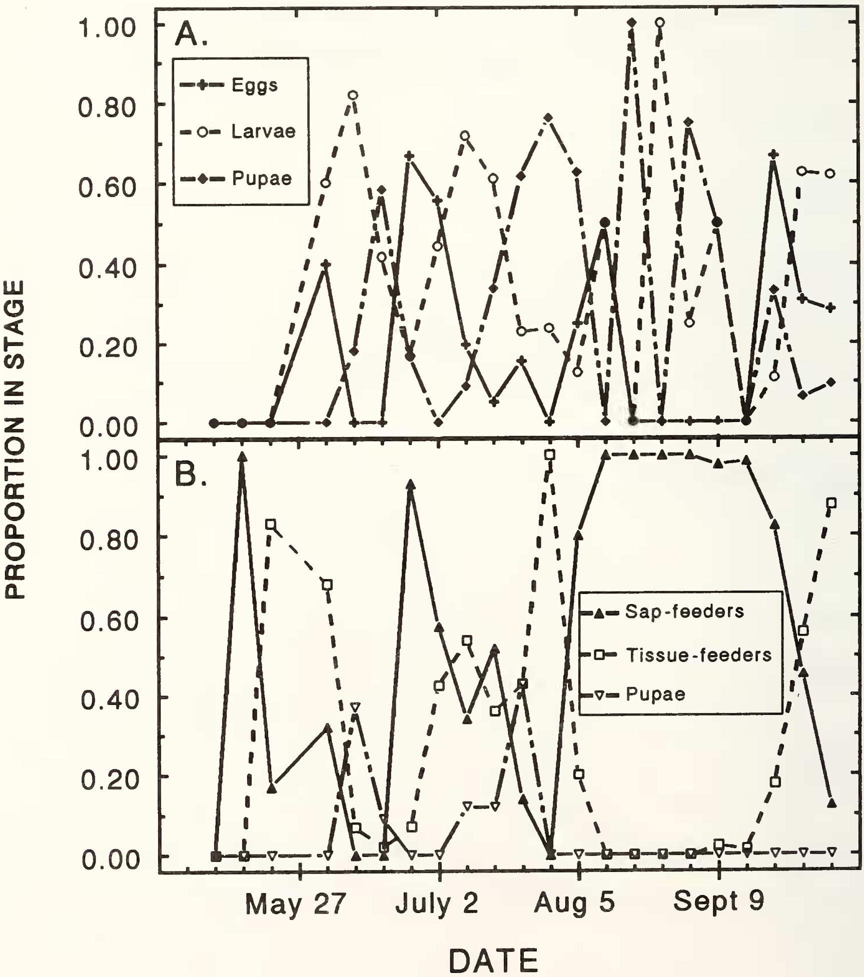

Figure 2. Proportion in each developmental stage: A = parasitoids *Pnigalio* and *Sympiesis*; B = leafminer *Phyllonorycter* determined from weekly samples May to October 1992 from orchard 1, Naramata.

Throughout both regions, *P. flavipes* remained the dominant parasitoid species over the three generations. Significantly ($p<0.05$) more *P. flavipes* emerged per 100 mines than did any other parasitoid species in all three generations. Despite its major role in the overwintering populations, *S. marylandensis* represented a small percent of the parasitoids in the first summer generation (Table 2, Fig. 1), increasing in its role as a parasitoid in the second generation.

The mean number of mines per leaf did not differ significantly ($p>0.05$) between the two regions in the second generation (Table 1). Mortality caused by host-piercing and oviposition of *P. flavipes* and *S. marylandensis* in the first generation was expected to be negatively correlated with the number of mines per leaf found in the second and third generations (Barrett and Brunner 1990, Cossentine and Jensen 1992, Van Driesche and Taub 1983). This was true only for the Osoyoos/Oliver region where the PIM in the tissue-feeding stage of the first generation was significantly negatively correlated (r=0.90-0.96, $p<0.05$) with the mines per leaf in the second generation over a two-week period.

The economic treatment threshold for leafminers in apple has been estimated at one sap-feeder per leaf for the first generation, two sap-feeders per leaf for the second generation provided that PIM is <30% in the first generation, and five sap-feeding mines per leaf in the third generation (Hoyt 1987). The only orchard sampled in this study that exceeded this treatment threshold in the second generation was in the Oliver/Osoyoos region. Leafminer counts in this orchard did not exceed thresholds in 1991. Despite finding six of the eight orchards with PIM above 30% in the first generation (Table 3), five of the eight orchards had exceeded the treatment threshold by the third generation (Table 1).

To explain the high 1992 *P. mespilella* populations, the warm weather early in the season in 1992 may have allowed the leafminers to establish large populations before *P. flavipes* could have a significant controlling effect. Unfortunately, neither emergence of adult *P. mespilella* nor the parasitoid species was assessed in the spring to judge if their emergences were synchronized. In Connecticut, adult *S. marylandensis* emerged two to four weeks before its host, *Phyllonorycter blancardella*, in the spring (Maier 1984). *S. marylandensis*, which made up about 50% of the overwintering parasitoid population, did not appear to influence *P. mespilella* control until the second and third generations by which time the leafminer populations were already above economic thresholds in some orchards. The developmental stages of the parasitoids appear to be in synchrony with those of the leafminer host in the first two summer generations (Fig. 2), with the parasitoids pupating just at the end of the *P. mespilella*'s first generation and the parasitoid eggs being laid at the beginning of generations two and three. The parasitoids appear to have two generations during the leafminers third (Fig. 2).

Parasitoid-induced-mortality increased with generation (Table 3), contrary to what was observed by Barrett and Brunner (1990) but they excluded mines judged to be from an earlier generation in their counts. We excluded mines but that were empty or contained an emerged pupa or a parasitoid hole. We do not believe that the dead larvae could be assigned to a particular generation consistently. The high PIM in the second and third generations in our study may be partially due to the accumulation of dead larvae from previous generations. However the PIM in the second and third generations was also high when compared to studies done in a similar fashion in the Okanagan valley from 1988 to 1990 (Cossentine and Jensen 1992). These high percentages indicate that a low number of live leafminers would be found in the 1992-93 overwintering mines. This must, at least in part, account for the low populations of *P. mespilella* observed in the summer of 1993.

We conclude that the percentage of *P. flavipes* and *S. marylandensis* in the overwintering leafminer population of 1991-92 did not differ between the orchards screened in Naramata and Oliver/Osoyoos, and could not explain the differences in the number of first generation mines in any of the eight orchards. We found *P. flavipes* to be the dominant parasitoid in the three summer generations. *S. marylandensis* did not start to participate within the complex, except at low levels in three orchards, until the second generation. Neither the *Eulophus* or *Cirrospilus* species previously found parasitizing the leafminer in the south Okanagan valley of British Columbia were found in the orchards studied.

The leafminer parasitoid complex is usually an efficient and effective biological control of the host populations. In 1992 leafminers exceeded economic thresholds in orchards in the Okanagan valley. Neither *P. flavipes* or *S. marylandensis* had a significant influence on intraseasonal leafminer increase. However, increasing season-long PIM had a dramatic impact on the number of leafminers entering overwintering diapause in the fall of 1992.

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REFERENCES

Barrett, B.A. 1988. The population dynamics of *Pnigalio flavipes* (Hymenoptera: Eulophidae), the major parasitoid of *Phyllonorycter elmaella* (Lepidoptera: Gracillariidae) in central Washington apple orchards. Ph.D. dissertation, Washington State University, Pullman.

Barrett, B.A. and J.F. Brunner. 1990. Temporal distribution of *Phyllonorycter elmaella* (Lepidoptera:Gracillariidae) and its major parasitoid *Pnigalio flavipes* (Hymenoptera: Eulophidae), in Washington apple orchards. Environ. Entomol. 19(2):362-369.

Barrett; B.A. and C.D. Jorgensen. 1986. Parasitoids of the western tentiform leafminer *Phyllonorycter elmaella* (Lepidoptera:Gracillariidae), in Utah apple orchards. Environ. Entomol. 15:635-641.

Cossentine, J.E. and L.B. Jensen. 1992. Establishment of *Phyllonorycter mespilella* (Hubner) (Lepidoptera:Gracillariidae) and its parasitoid, *Pnigalio flavipes* (Hymenoptera:Eulophidae), in fruit orchards in the Okanagan and Similkameen valleys of British Columbia. J. Entomol. Soc. Brit. Columbia. 89:18-24.

Doganlar, M. and B. Bierne. 1980. Parasites of *Phyllonorycter elmaella* (Lep.: Gracilariidae) on apple in the Vancouver district, British Columbia. Can. Entomol. 112:314.

Hoyt, S. 1983. Biology and control of the western tentiform leafminer. Proc. WA Hort. Assoc. 79:115-118.

Hoyt, S. C. 1987. Impact and thresholds of the leafminer on apple. pp. 7-9. In: Leafminer short course. Washington State University, College of Agriculture and Home Economics, Cooperative Extension Service. 17 p.

Maier. C. T. 1984. Abundance and phenology of parasitoids of the spotted tentiform leafminer, *Phyllonorycter blancardella* (Lepidoptera:Gracillariidae), in Connecticut. Can. Entomol. 116:443-449.

SAS Institute. 1985. SAS User's Guide: Statistics, Version 5 edition. SAS Institute, Cary, N.C. 956 pp.

Van Driesche, R. G. 1983. Meaning of "Percent Parasitism" in studies of insect parasitoids. Environ. Entomol. 12(6):1611-1622.

Van Driesche, R. G. and G. Taub. 1983. Impact of parasitoids on *Phyllonorycter* leafminers infesting apples in Massachusetts, U.S.A. Prot. Ecol. 5:303-317.